REMARKS

This response is filed in reply to the Office Action mailed August 26, 2003. Claims 8-19 has been canceled. Claims 1-7 have been amended. New claims 30-46 have been added. Support for the amended claims and new claims can be found throughout the specification: e.g., in the original claims, in the Examples, and at pages 9-11.

Applicants note that non-elected claims 9-29 have been withdrawn from consideration. Applicants will request that withdrawn claims 20, 22, 24, 26, and 28 be rejoined pursuant to MPEP §821.04 once the elected claims are deemed allowable.

No new matter has been added. Claims 1-7 and 30-47 are pending and at issue. Applicants request reconsideration of the pending claims, as amended.

I. REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Written Description

Claim 8 stands rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. This rejection is moot in view of the cancellation of claim 8. To the extent that it may be applied to any of the present claims that, like claim 8, do no include a functional limitation (i.e., claims 31-33, 35-40, and 43-46), the rejection is traversed. Applicants maintain that the structure of every species within claim 8 and within claims 31-33, 35-40, and 43-46 is fully and explicitly described, and thus the rejection was improper. Function is simply one way to describe a protein—it is not required when other characteristics, such as structure, are provided. The polypeptides encompassed by these claims are fully described in terms of amino acid sequence. Withdrawal of the rejection is respectfully requested.

Enablement

Claim 8 stands rejected under 35 USC §112, first paragraph, because the specification, while being enabling for mutants of SEQ ID NO:2 having formate dehydrogenase activity, does

not reasonably provide enablement for mutants having unknown function. This rejection is moot in view of the cancellation of claim 8. Applicants traverse this rejection as it may apply to the pending claims that, like claim 8, do not recite a function: i.e., claims 31-33, 35-40, and 43-46. As the specification discloses the generation and testing of the polypeptides covered by these claims (see Examples 3-22), there is no question that applicants have taught how to make and use the claimed proteins. The rejection is therefore unwarranted. Accordingly, Applicants respectfully request withdrawal of the §112, first paragraph rejection.

II. REJECTIONS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-7 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter that the Applicants regard as the invention. Specifically, the Examiner finds the term "strong" to be indefinite as it is allegedly unclear what range of activities is considered to meet this requirement. Applicants note that the specification explicitly defines the phrase "strong activity of formate dehydrogenase" as meaning that the enzyme activity is "at least 70% or higher, for example, 80% or higher, preferably 90% or higher, more preferably 95% or higher in the presence of an organic solvent when compared with the enzyme activity in the absence of organic solvent." (See page 9, lines 7-10). Thus, not only would one skilled in the art readily comprehend the metes and bounds of the claims, but he/she could also easily determine whether a particular species falls within or outside the scope of the claim through routine experimentation. Nevertheless, in order to expedite prosecution, Applicants note that the new claims do not employ the language that the office action describes as "confusing". Accordingly, Applicants request withdrawal of this rejection.

III. REJECTION UNDER 35 U.S.C. §103

Claims 1-8 stand rejected under 35 U.S.C. §103 as allegedly obvious over Galkin et al. in view of Slusarczyk et al. and Tishkov et al. This rejection is moot with respect to canceled claim 8. Applicants traverse this rejection as it may be applied to the pending claims.

The Office Action alleges that Galkin et al. teach a mutant formate dehydrogenase of *Pseudomonas sp.* that results from the substitution of Cys-255 with a serine residue. The Office Action further alleges that Cys-255 of the *Pseudomonas sp.* formate dehydrogenase corresponds to Cys-256 of SEQ ID NO:2, the *Mycobacterium vaccae* enzyme of the instant invention. The Office Action acknowledges that Galkin et al. fail to teach mutant formate dehydrogenases having substitutions at positions 6 and 146 of SEQ ID NO:2.

To cure the deficiencies of Galkin et al., the Office Action cites to Slusarczyk et al. and Tishkov et al. as teaching mutant formate dehydrogenases wherein the cysteine residues are replaced to increase the enzyme's stability. According to the Office Action, Slusarczyk et al. discloses the substitution of "all" the cysteine residues with aliphatic amino acids, such as alanine and valine, to alleviate any thiol-coupled inactivation of the enzyme, thereby increasing the enzyme's stability. In addition to the Cys-255-Ser modification of the *Pseudomonas* enzyme, Tishkov et al. allegedly teaches that Cys-5 (which corresponds to Cys-6 of SEQ ID NO:2) "is involved in the stability of the enzyme." Office Action at page 7. The Office Action concludes that, in light of the teachings of Slusarczyk and Tishkov, it would have been obvious to substitute serine, alanine or valine residues for the cysteine residues of the formate dehydrogenase of Galkin et al. to prevent thiol-coupled inactivation of the enzyme, thereby increasing its stability.

In order to address the obviousness rejection as it may apply to the presently pending claims, it is useful to divide the claims into three overlapping groups, discussing each separately.

Applicants first focus on claims 4-7 and 30-46 as a group. Each of these claims is drawn to a polypeptide comprising the sequence of SEQ ID NO:2 (the sequence of *Mycobacterium vaccae* formate dehydrogenase (McFDH)) in which Cys-256 of SEQ ID NO:2 (and in some of these claims, other specified Cys residues as well) is substituted with a different residue. Galkin et al. cloned and studied McFDH, observing that it differed from the formate dehydrogenase of *Pseudomonas sp.* (PsFDH) at two positions, neither of which is a Cys: Ile-35 of McFDH (Thr in PsFDH) and Glu-61 of McFDH (Lys in PsFDH). Galkin et al. also observed that the thermostability of wildtype McFDH was "a little lower" than that of wildtype PsFDH (see abstract). By substituting various residues for Glu-61 of McFDH, Galkin et al. were able to

increase the thermostability of the McFDH enzyme somewhat, compared to wildtype. Galkin et al. did not experiment with replacing any of the Cys residues of McFDH. They did, however, mention that Tishkov et al. had tried replacing Cys-255 of PsFDH with Ser, and had found the resulting mutant to be less thermostable than wildtype PsFDH. Given the focus of Galkin et al. on thermostability, it cannot be said to have been obvious in view of these two references to make the corresponding substitution in McFDH. Quite to the contrary: one of ordinary skill in the art would have read Tishkov et al. as teaching away from making a similar Cys-to-Ser substitution in McFDH, and thereby potentially decreasing the thermostability of this enzyme (already lower than that of PsFDH) even more. In fact, the Office Action has not established any reason why one of ordinary skill would have selected McFDH over PsFDH in the first place, given the teachings of Galkin et al. about the superior thermostability of PsFDH. Nor does the Office Action explain why one of ordinary skill would have ignored the teachings of Galkin et al. about improving the thermostability of McFDH by modifying the Glu-61 residue, choosing to leave that residue intact and alter an untested Cys residue instead. Neither Tishkov et al. nor Slusarczyk et al. contradicts these teachings of Galkin et al. regarding thermostability, nor teaches any advantage of the McFDH enzyme as starting material. And certainly nothing in the art gave any inkling that mutating Cys-256 of McFDH would yield an enzyme with the particularly advantageous characteristic discovered by applicants: increased activity in the presence of organic solvent. An important commercial use of formate dehydrogenase is for regenerating NADH from its oxidized form, NAD+, during enzymatic production of an alcohol ((S)-4-halo-3-hydroxybutyrate ester) from a ketone (4-haloacetoacetate ester) using carbonyl reductase. Carbonyl reductase requires NADH as a coenzyme for the enzymatic conversion of this ketone to the corresponding alcohol, producing NAD+ as a by-product. Because it would be costly to keep adding fresh NADH to the reaction as it is consumed, a second enzyme, formate dehydrogenase, is included in the reaction mixture to regenerate NADH from the NAD+. However, as described in the present application, formate dehydrogenase is notoriously unstable when included in this reaction. Applicants have discovered that an important reason for the instability of formate dehydrogenase in this reaction is its inactivation in the presence of organic

solvents such as the 4-haloacetoacetate ester used as substrate for the carbonyl reductase reaction. As detailed in the specification at page 10 and Example 20 on pages 39-40, mutating McFDH at Cys-256 quite surprisingly produces a mutant enzyme with vastly improved resistance to organic solvent. This result was not predicted by the prior art. For this reason, and all of the other reasons outlined above, claims 4-7 and 30-46 are not obvious over the cited art.

Next, Applicants address claims 1-7 and 42-46, each of which claims a polypeptide having a mutation at the position corresponding to Cys-146 of SEQ ID NO:2. None of the cited references alone or in combination teaches or suggests modification of Cys-146 of any formate dehydrogenase, much less of McFDH (SEQ ID NO:2). Thus, there is simply no motivation in the art to arrive at the invention of any of claims 1-7 and 42-46.

The Office Action attempts to overcome this clear-cut deficiency in the art by asserting that Slusarczyk et al. teach mutagenizing "all" cysteine residues to increase the enzyme's stability. Slusarczyk et al. experimented with a *Candida boidinii* formate dehydrogenase (CbFDH) that is 54% identical to PsFDH (p.1285, col.2) and thus presumably about 54% identical to McFDH as well. Unlike McFDH and PsFDH, which possess a total of seven Cys residues each, Slusarczyk et al.'s enzyme has only two Cys residues. One of these, Cys-262, aligns with Cys-288 of PsFDH (and consequently of McFDH). (See page 1286, col.1, and also Fig.2 of Slusarczyk et al.) The other Cys in CbFDH, Cys-23, has no Cys counterpart in PsFDH or McFDH; instead, the aligned position is occupied by Ser in these bacterial enzymes (see Fig.2 of Slusarczyk et al.). Slusarczyk et al. produced a number of CbFDH mutants in which one or both Cys residues were replaced with other residues. Tests on these mutant enzymes showed that replacing Cys-23 dramatically increased the stability of the enzyme under various experimental conditions, while replacing Cys-262 did not. In fact, under some conditions, replacing Cys-262 actually decreased the activity relative to wildtype (see, e.g., Figs. 9 and 11), and replacing both Cys-23 and Cys-262 decreased the activity relative to replacing Cys-23 alone (see, e.g., Figs. 10 and 11). If one can extrapolate anything at all from the teachings of Slusarczyk et al. to other formate dehydrogenases, it would be something to the effect that replacing a Cys corresponding to Cys-23 of CbFDH might produce an enzyme that is more

stable under certain conditions, but replacing a Cys corresponding to Cys-262 of CbFDH would not—and in fact can be detrimental. Since McFDH does not have a Cys residue corresponding to Cys-23, and the present claims do not specify replacing the McFDH Cys residue corresponding to Cys-262 of CbFDH, Slusarczyk et al. appears to have no relevance whatsoever to the present claims—other than, perhaps, a general teaching that results will vary markedly depending on the position of the Cys residue that is replaced. This reference, whether alone or in combination with the other cited references, certainly cannot be taken as a teaching that one can usefully mutagenize *all* Cys residues of *all* formate dehydrogenases. In fact, it teaches quite the contrary. Even more far-fetched is the notion that the cited references suggest one should target Cys-146, *in particular*, of McFDH, *in particular* (or, for that matter, either of the other Cys residues specified in the present claims). Furthermore, nothing in any of the cited references suggests that substituting Cys-146 of McFDH would produce the surprising activity in the presence of organic solvents that applicants have observed—a problem that is addressed nowhere in the cited art. As this amply demonstrates that claims 1-7 and 42-46 are nonobvious over the cited art, withdrawal of the rejection is respectfully requested.

The last grouping of claims (which overlaps with the previous two groupings above) includes claims 4-7 and 34-46. Each of these claims falls into one or both of the two groupings above, and so is patentable over the art for the reasons discussed above. Applicants address them again here in order to point out an additional basis for their patentability: the fact that they require that <u>at least two</u> of the specified Cys residues (Cys-6, Cys-146, and Cys-256) be modified. Nowhere does the cited art suggest this. Galkin et al. studied only non-Cys residues in McFDH. Slusarczyk et al. used a distantly related yeast enzyme, and focused on Cys residues that do not correspond to any of the three specified by the present claims. As discussed above, Tishkov et al. found that replacing a Cys in PsFDH corresponding to McFDH Cys-256 produced a protein with decreased thermostability. They did not try replacing other Cys residues in PsFDH, and certainly provided no reason to replace even Cys-256 in McFDH, which (according to Galkin et al.) is even less thermostable than wildtype PsFDH. Tishkov et al. mentioned on

page 979 that Cys-5 of PsFDH (corresponding to Cys-6 of McFDH) is likely to be "essential for enzyme activity," providing a reason not to alter it if preserving activity is the goal. Thus, there is no motivation in the art to make the claimed mutants. If anything, one would be dissuaded from doing so by the results reported in the art. Finally, applicants again note the surprising results discussed above, which apply to the double and triple mutants embodied in this last grouping of claims. The Examiner is pointed to the examples in the specification reporting results with several double and triple mutants of the invention (see, e.g., Tables 2-4). These unexpected results are powerful evidence of nonobviousness, and must be taken into account when assessing the patentability of the claims over the prior art.

In view of the above the above discussion, the amendments to claims 1-7, and the presentation of new claims 30-46, Applicants respectfully request that the rejections under 35 U.S.C. §103 be withdrawn.

In summary, for the reasons set forth herein, Applicants maintain that claims 1-7 and 30-46 clearly and patentably define the invention. Applicants request that the Examiner reconsider and withdraw the various grounds for rejection set forth in the Office Action. If the Examiner would like to discuss any of the issues raised in the Office Action, Applicants' representative can be reached at (617) 542-5070. A petition for a two-month extension of time and a check for the requisite fee are enclosed. Also enclosed is a check for $674.00 to cover the excess claims fee. Please charge any additional fees, or make any credits, to Deposit Account No. 06-1050.

Respectfully submitted,

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